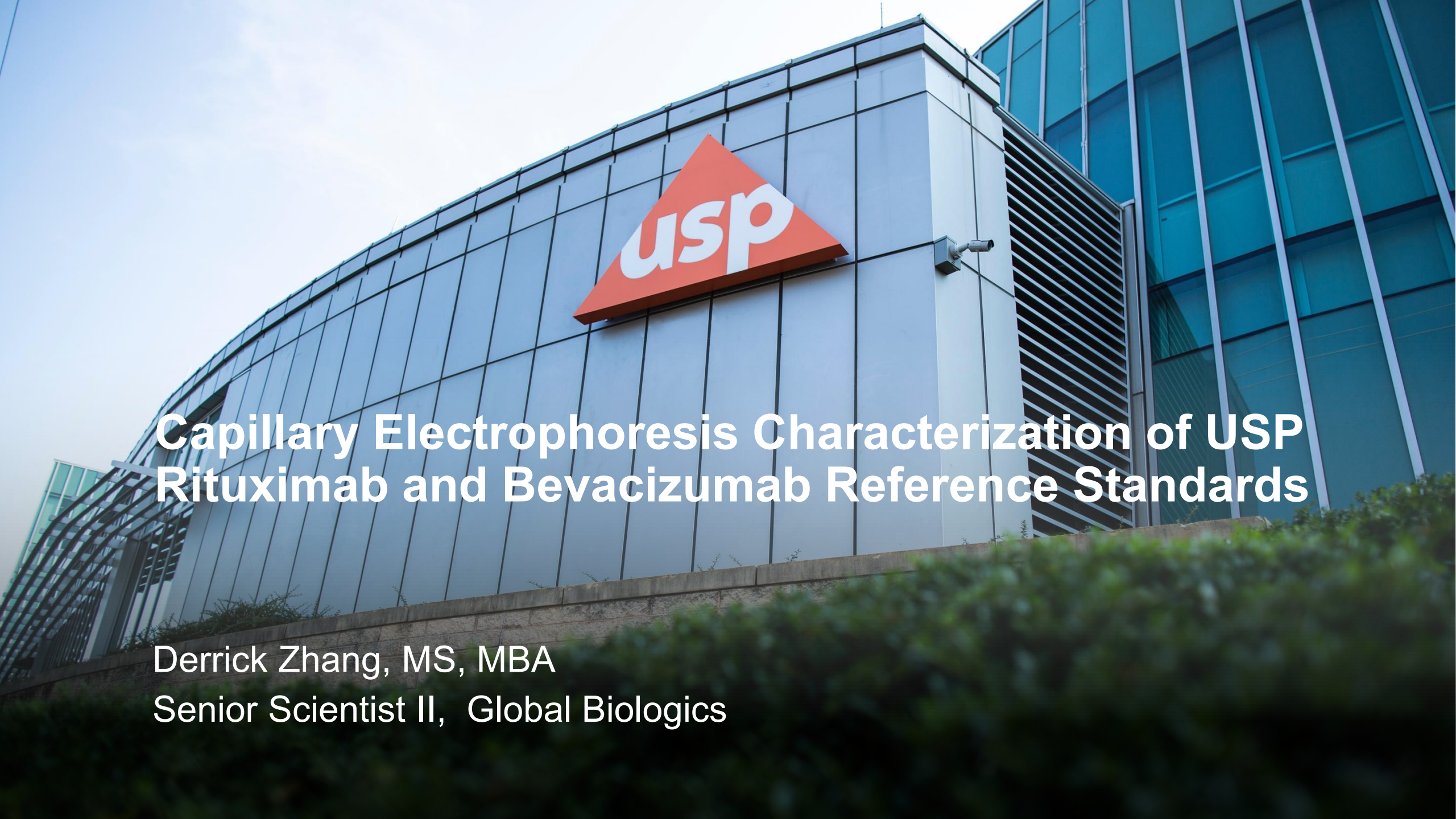
Welcome



The standard of trust



Capillary Electrophoresis Characterization of USP Rituximab and Bevacizumab Reference Standards

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Agenda

- Introduction
- USP Emerging Standards
- CE Characterization
- Conclusion





Introduction

Global Trends in Biologics & Biosimilars

Rapid Growth of Biologics Market

- Biologics dominate top-selling drugs
- Expansion beyond oncology → immunology, rare diseases

Adoption of Biosimilars

- Increasing regulatory approvals globally
- Cost reduction + access driver
- Still uneven adoption across regions

Evolving Regulatory Paradigms

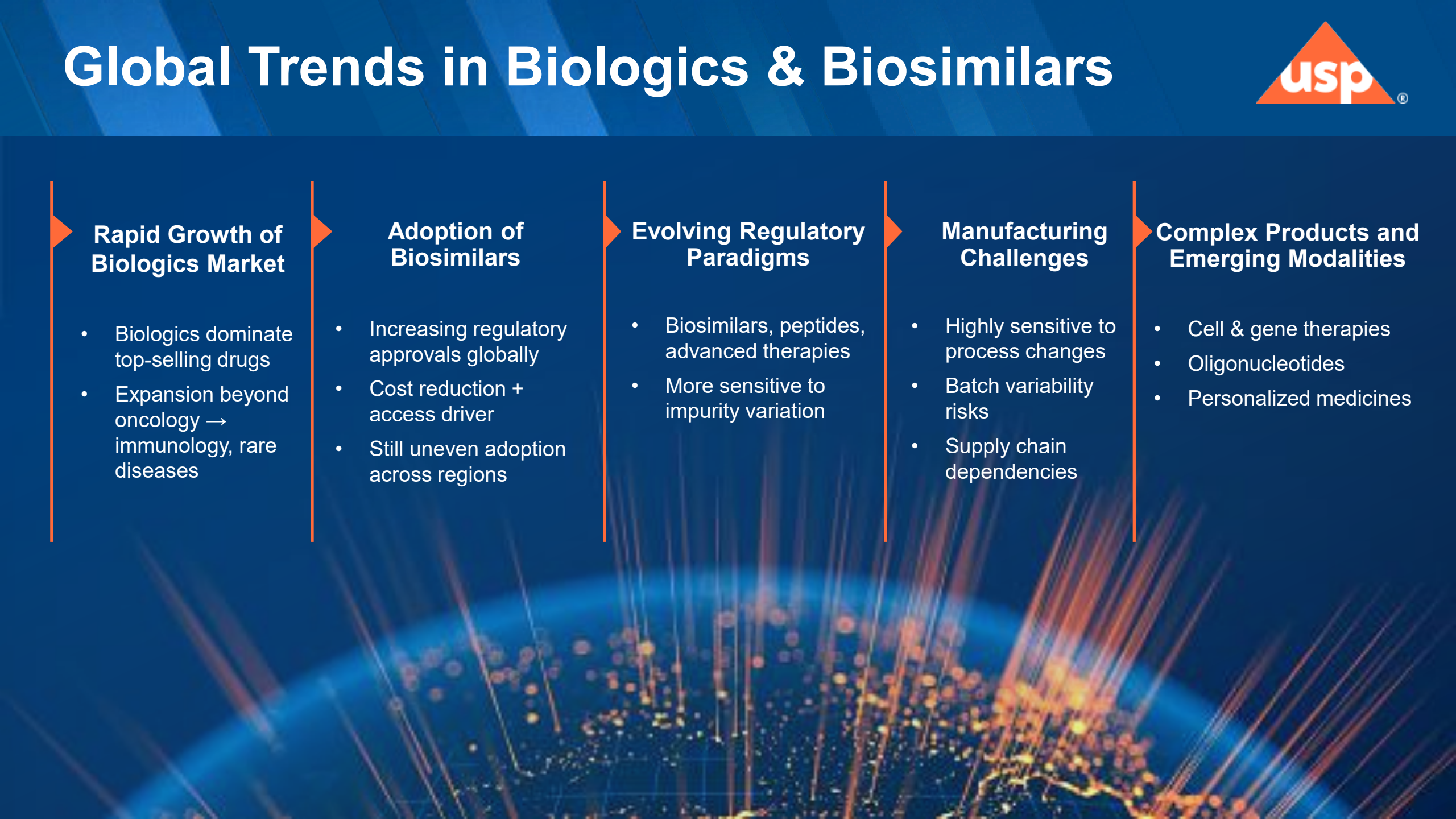
- Biosimilars, peptides, advanced therapies
- More sensitive to impurity variation

Manufacturing Challenges

- Highly sensitive to process changes
- Batch variability risks
- Supply chain dependencies

Complex Products and Emerging Modalities

- Cell & gene therapies
- Oligonucleotides
- Personalized medicines



USP Standards



- **Documentary Standards**
  - **Monographs** provide product-specific requirements for quality assessment
  - **Procedural general chapters** (numbered under 1000)
    - Validated methods
    - May or may not be referenced in monograph
  - **Informational general chapters** (numbered 1000 to 1999)
    - General information and best practices

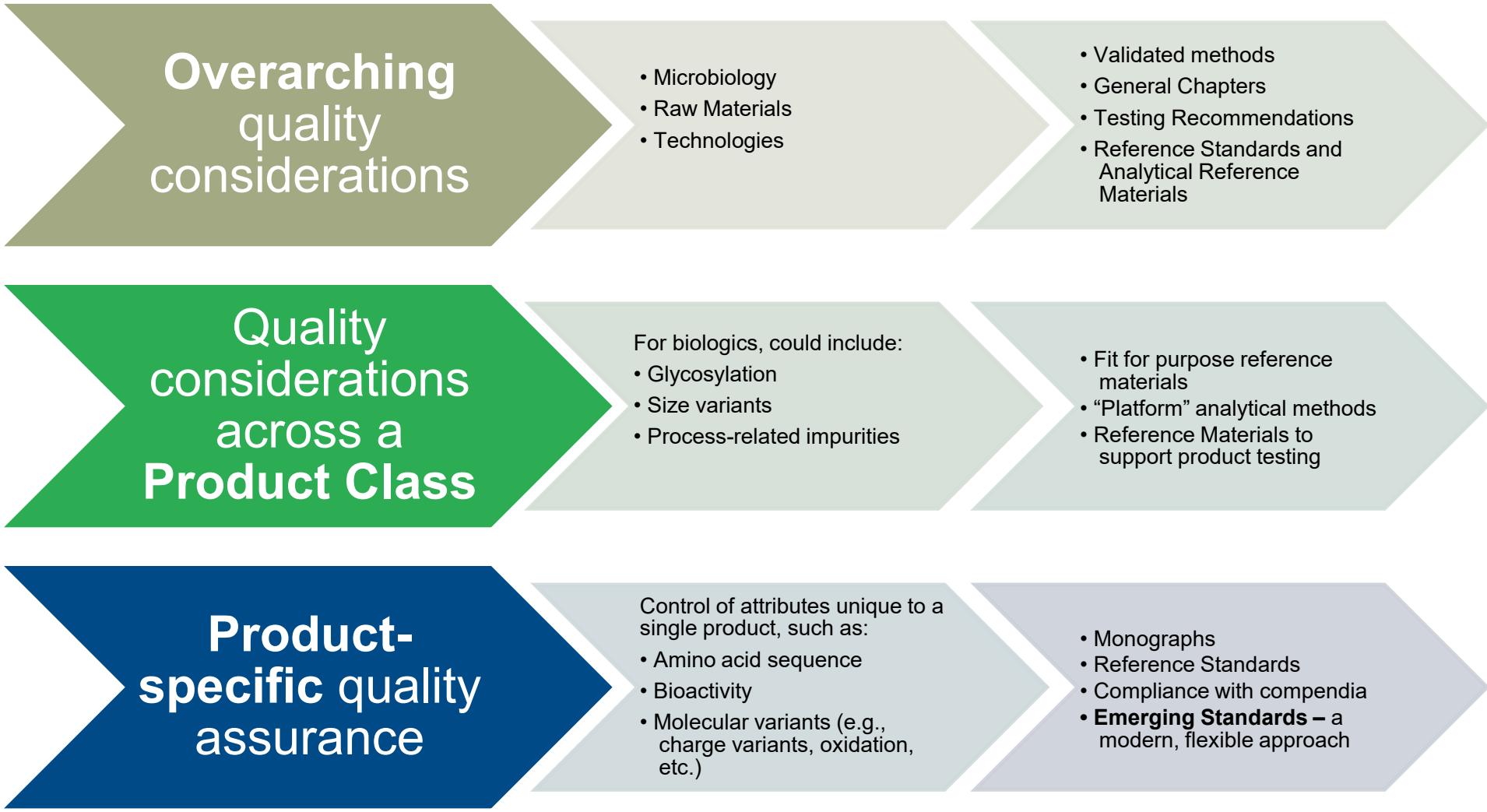


- **Physical Reference Materials**
  - **Reference Standards** provide traceable standards to demonstrate broad-based acceptability of procedures and are often associated with a procedural chapter
  - **Analytical Reference Materials (ARM)** are non-compendial and well characterized
    - For research and development use in a variety of analytical applications
    - Used for method development, trouble shooting or investigations throughout product lifecycle



USP Emerging Standards

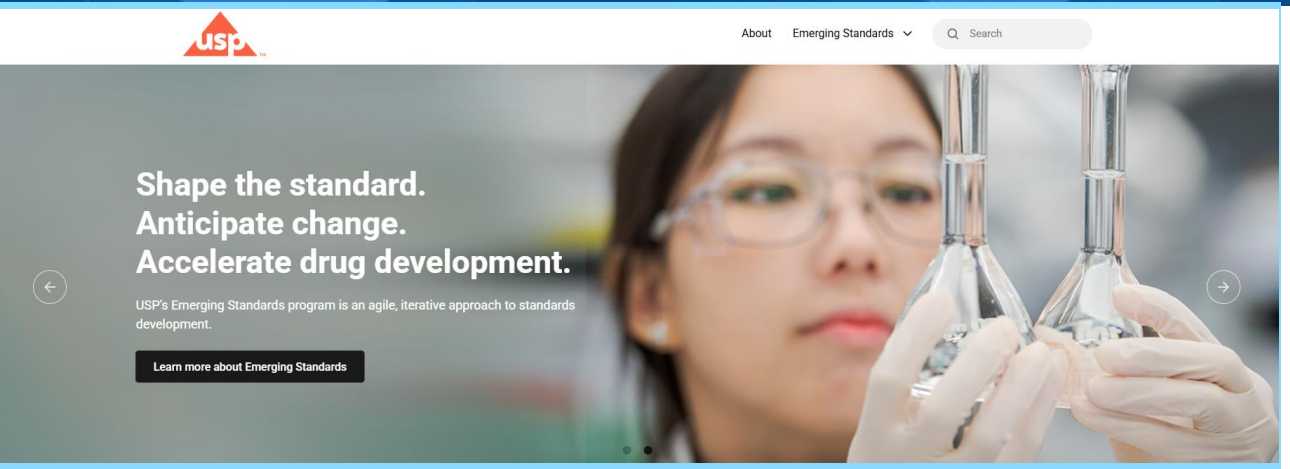
USP Approaches to Advancing Biologics Quality across Modalities



Product-Class Standards and Tools for mAbs

	Quality Attribute	Test	USP Chapter	Reference Standard or Material
Validated Methods	**Size Variants** (aggregation, fragmentation)	Size-exclusion chromatography (with HPLC or UPLC) CE-SDS	<129> *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies*	USP Monoclonal IgG System Suitability RS
	Protein Concentration	UV - native, denaturing, VPE* BCA, Bradford, Lowry Amino Acid Analysis	<507> *Protein Determination Procedures*	USP BSA for Protein Quantitation RS
	Glycosylation	Oligosaccharide and monosaccharide analysis by HPLC or CE	<212> *Oligosaccharide Analysis* <210> *Monosaccharide Analysis* <129> *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies*	USP Oligosaccharide System Suitability Mixture A RS N-acetylneuraminic acid RS N-glycolylneuraminic acid RS
	Host Cell DNA	qPCR	<509> *Residual DNA Testing*	USP CHO Genomic DNA RS USP E. coli Genomic DNA RS
Best Practices	**Multi-attribute Method**	Peptide-based mass spectrometry workflow	<1060> *Mass Spectrometry Based Multi-Attribute Method for Therapeutic Proteins*	USP mAb 001 RS (or mAb 002, 003, 004) Digested mAb in development
	Host Cell Protein	ELISA Mass spectrometry-based identification and quantitation	<1132> *Residual Host Cell Protein Measurement in Biopharmaceuticals* <1132.1> *Residual Host Cell Protein Measurement in Biopharmaceuticals by Liquid Chromatography-Mass Spectrometry*	CHO Null Cell HCCF ARM PLBL2 ARM CHO HCP Heavy Peptide ARMs for HCPs of concern

Emerging Standards

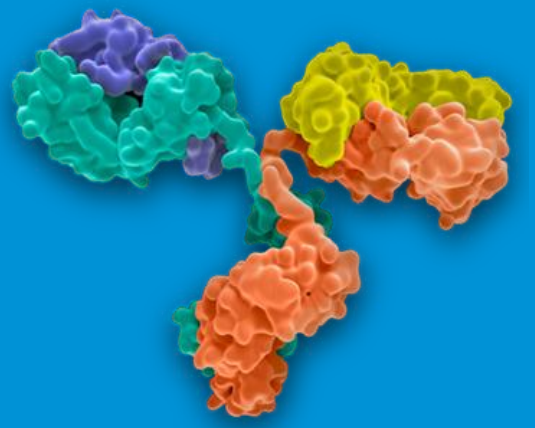


USP's Emerging Standard platform is an agile, iterative approach to documentary standard development, where a potential standard is shared with the community to stimulate dialogue and engagement prior to any compendial consideration

https://emergingstandards.usp.org/

- Emerging standards can include:
  - Biologics
  - Digital standards
  - Small molecules
- Emerging Standards are not official standards
  - They may evolve into official USP standards, with stakeholder input and support
- We welcome input
  - Input received at this early stage can significantly influence and shape an emerging standard's development trajectory

Example: Emerging Standard for Rituximab



Identification

- Biological assay
- Peptide mapping

Biological Activity

- ADCC Cell Based Assay
- CDC Cell Based Assay

Protein Content

- <507> *Protein Determination Procedures*

Glycan Profile

- Specific N-Glycan profiling
- <129> *Analytical Procedures for mAbs*
- <212> *Oligosaccharide Analysis*
- <210> *Monosaccharide Analysis*

Charge Variants

- Cation Exchange Chromatography
- cIEF
- icIEF

Size Variants

- <129> *Analytical Procedures for mAbs*
  - Size Exclusion Chromatography
  - CE-SDS (reducing and non-reducing)

Specific Tests

- *<61> Microbial Enumeration Tests*
- *<71> Sterility Tests*
- *<85> Bacterial Endotoxins tests*
- Host Cell Proteins
- *<509> Host Cell DNA*
- Protein A

Product Specific Reference Standards

Rituximab Reference Standard

- A chimeric murine/human IgG1 antibody designed to target the CD20 antigen on B cells
- USP Certificate includes:
  - Concentration by SoloVPE
  - Intact mass by LC-MS
  - Aggregates by SE-HPLC and SE-UHPLC
  - CE-SDS (reduced and non-reduced)
  - Charge variants by cIEF and icIEF
  - LC-MS (MAM)-based analysis of PTMs
  - N-glycan profiling by CE-LIF and HILIC-FLR-MS
  - Potency Assays:
    - ADCC reporter gene assay
    - CDC bioassay

Bevacizumab Reference Standard

- A humanized IgG1 antibody targeting vascular endothelial growth factor (VEGF)
- USP Certificate includes:
  - Concentration by SoloVPE
  - Intact mass by LC-MS
  - Aggregates by SE-HPLC and SE-UHPLC
  - CE-SDS (reduced and non-reduced)
  - Charge variants by cIEF and icIEF
  - LC-MS (MAM)-based analysis of PTMs
  - N-glycan profiling by CE-LIF and HILIC-FLR-MS
  - Potency Assay:
    - VEGF reporter gene assay



CE Characterization:

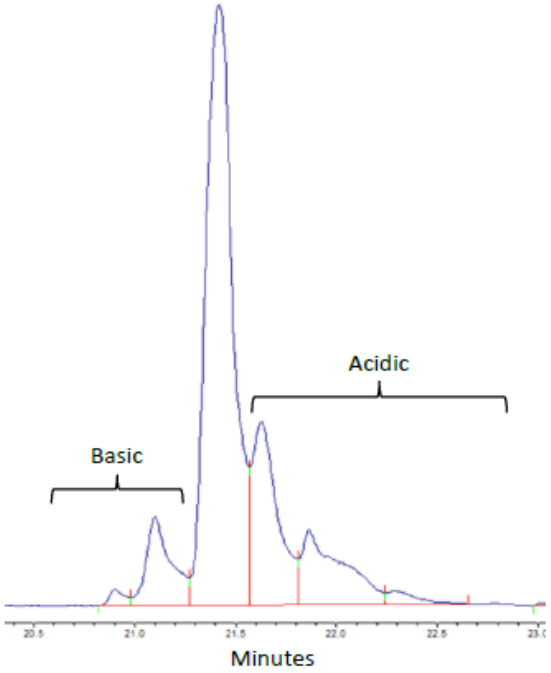
USP Rituximab RS and USP Bevacizumab RS

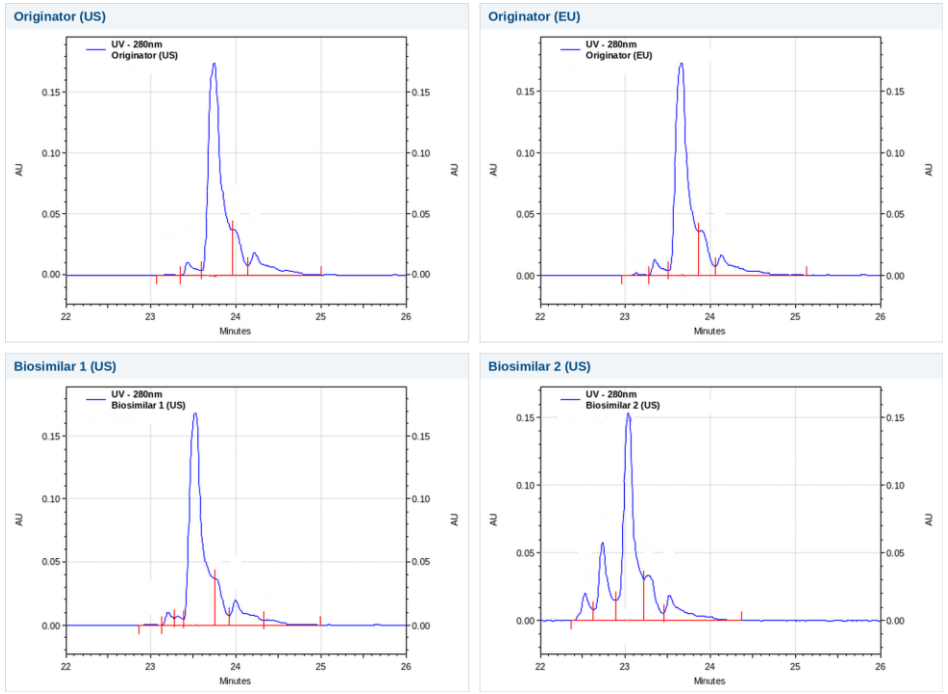
Materials and Testings

- Materials:
  - Rituximab
    - 2 Originator: one lot each from US and EU
    - 2 Biosimilars: US approved
  - Bevacizumab
    - 2 Originator: one lot each from US and EU
    - 2 Biosimilars: US approved
- Testing Performed:
  - CE-SDS: method performed according to USP <129>
  - cIEF: method performed according to Emerging Standard
  - icIEF: method performed according to Emerging Standard
  - CZE: method performed according to AB Sciex CZE Protocol
  - CEX: method performed according to Emerging Standard

cIEF: Rituximab

USP Rituximab RS



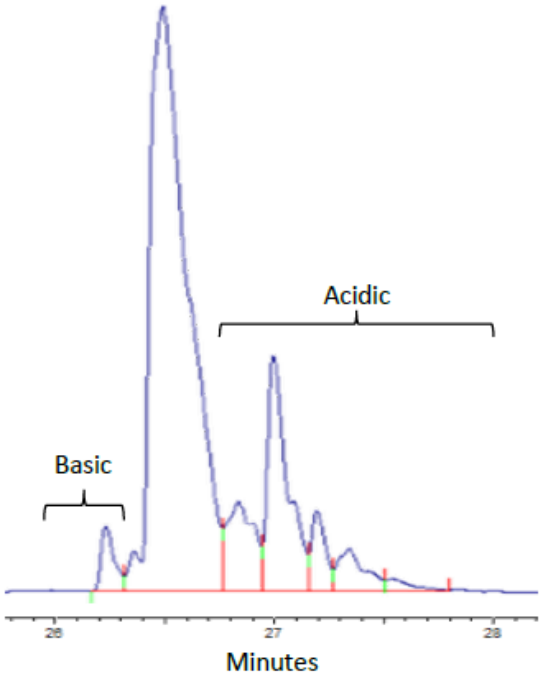


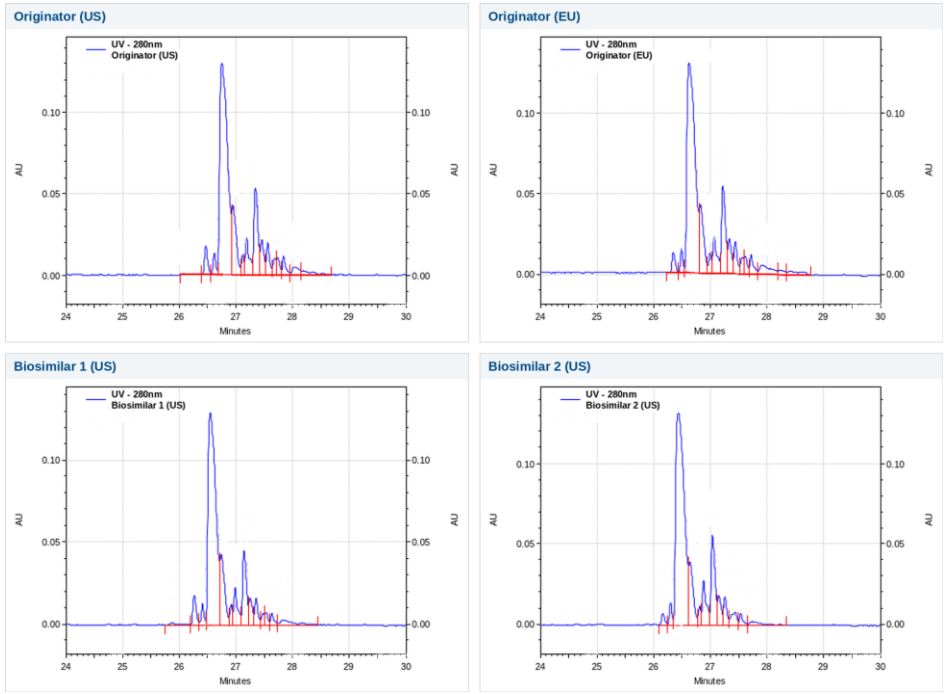
Sample	Main peak pI	Acidic (%)	Main (%)	Basic (%)
USP Rituximab RS	**9.2**	**32**	**60**	**8**
Originator (US)	9.4	23	74	4
Originator (EU)	9.4	22	73	4
Biosimilar 1 (US)	9.3	25	71	4
Biosimilar 2 (US)	9.4	22	54	25

1. **RS data shown here are certificate values**
2. **For detailed method please visit: https://emergingstandards.usp.org/**

cIEF: Bevacizumab

USP Bevacizumab RS



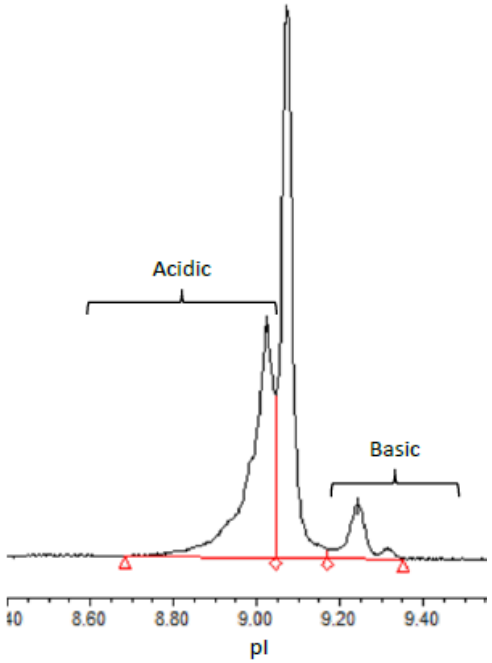


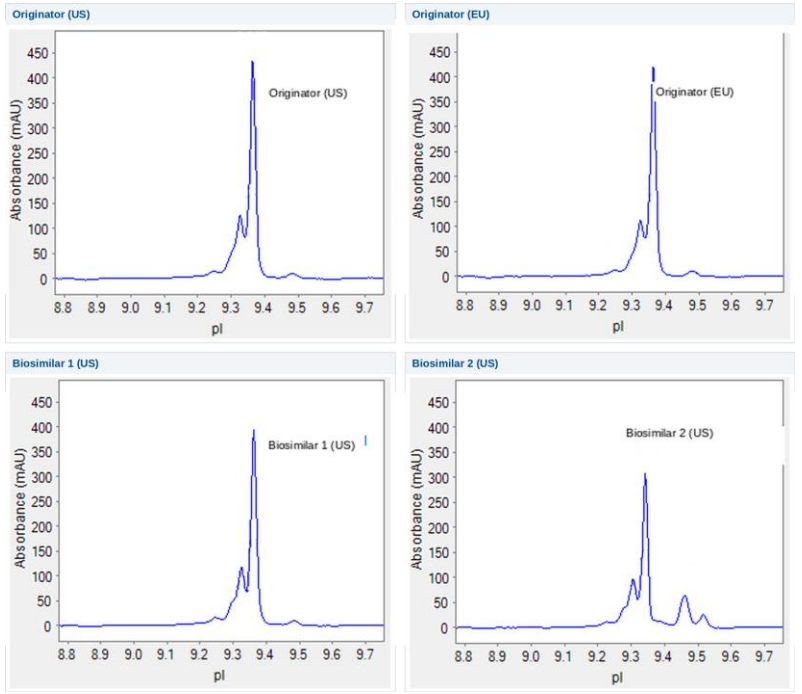
Sample	Main peak pI	Acidic (%)	Main (%)	Basic (%)
USP Bevacizumab RS	**7.8**	**30**	**65**	**4**
Originator (US)	8.0	33	62	5
Originator (EU)	8.0	34	62	4
Biosimilar 1 (US)	8.0	29	66	6
Biosimilar 2 (US)	8.0	30	67	3

1. **RS data shown here are certificate values**
2. **For detailed method please visit: https://emergingstandards.usp.org/**

iclEF: Rituximab

USP Rituximab RS



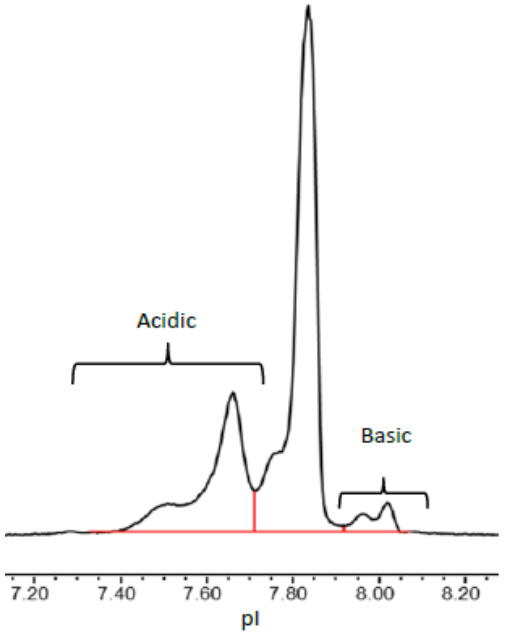


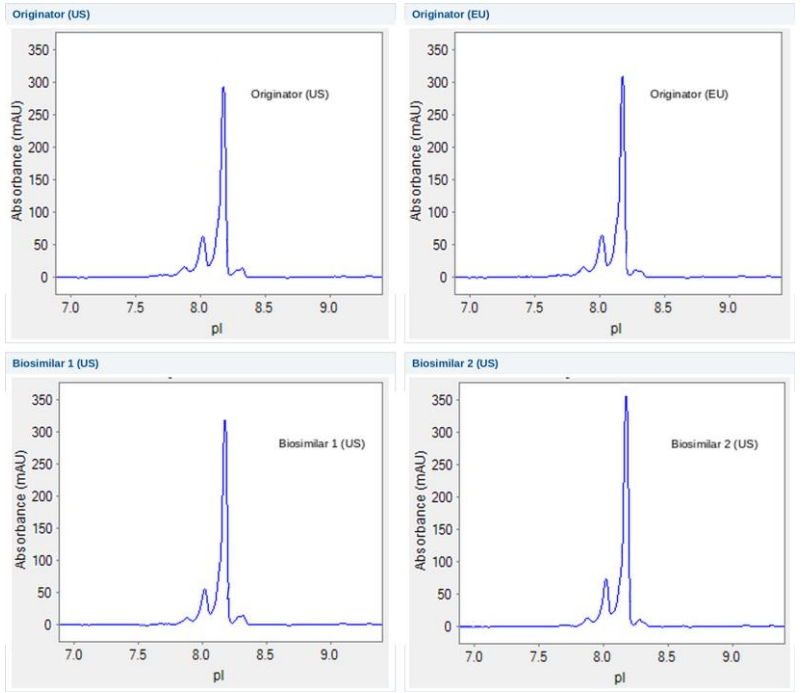
Sample	Main Peak pI	Acidic (%)	Main (%)	Basic (%)
USP Rituximab RS	**9.2**	**38**	**54**	**8**
Originator (US)	9.4	34	64	2
Originator (EU)	9.4	31	66	3
Biosimilar 1 (US)	9.4	35	62	2
Biosimilar 2 (US)	9.3	29	50	21

1. **RS data shown here are certificate values**
2. **For detailed method please visit: https://emergingstandards.usp.org/**

icIEF: Bevacizumab

USP Bevacizumab RS



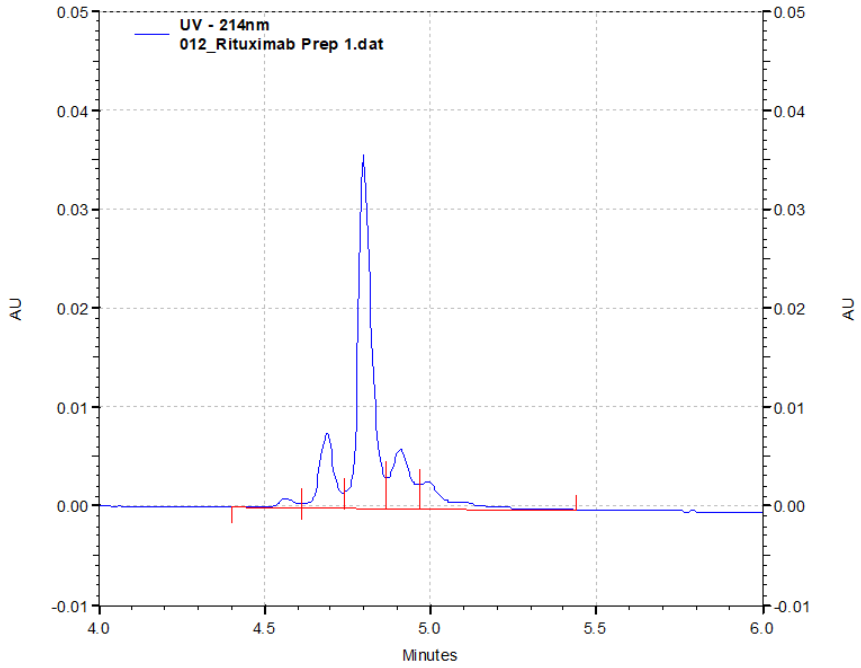


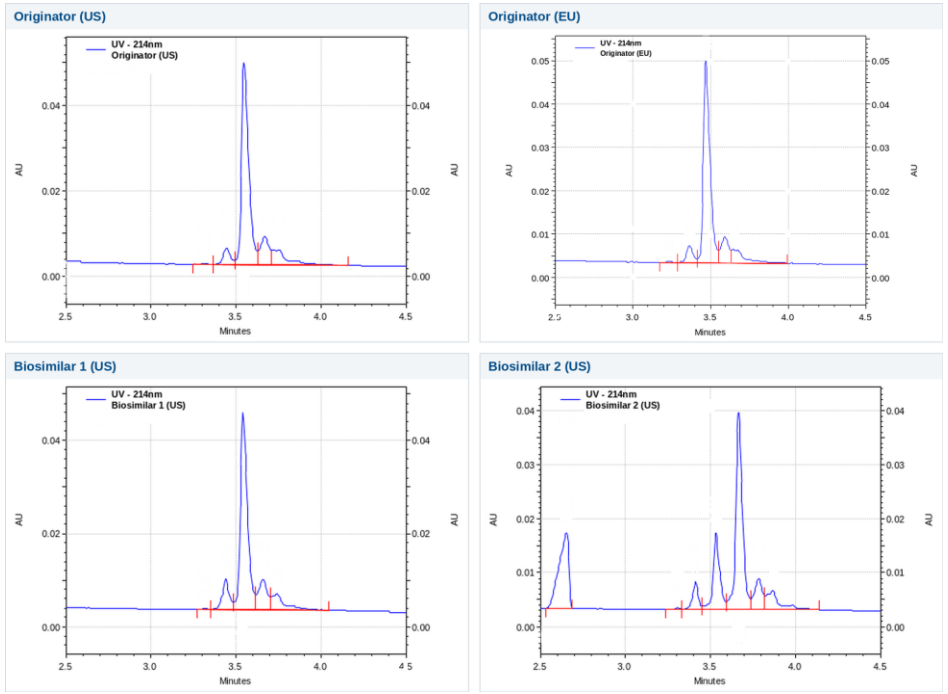
Sample	Main peak pI	Acidic (%)	Main (%)	Basic (%)
USP Bevacizumab RS	**7.9**	**28**	**67**	**4**
Originator (US)	8.2	27	68	5
Originator (EU)	8.2	27	70	4
Biosimilar 1 (US)	8.2	22	72	5
Biosimilar 2 (US)	8.2	24	74	3

1. **RS data shown here are certificate values**
2. **For detailed method please visit: https://emergingstandards.usp.org/**

CZE: Rituximab

USP Rituximab RS



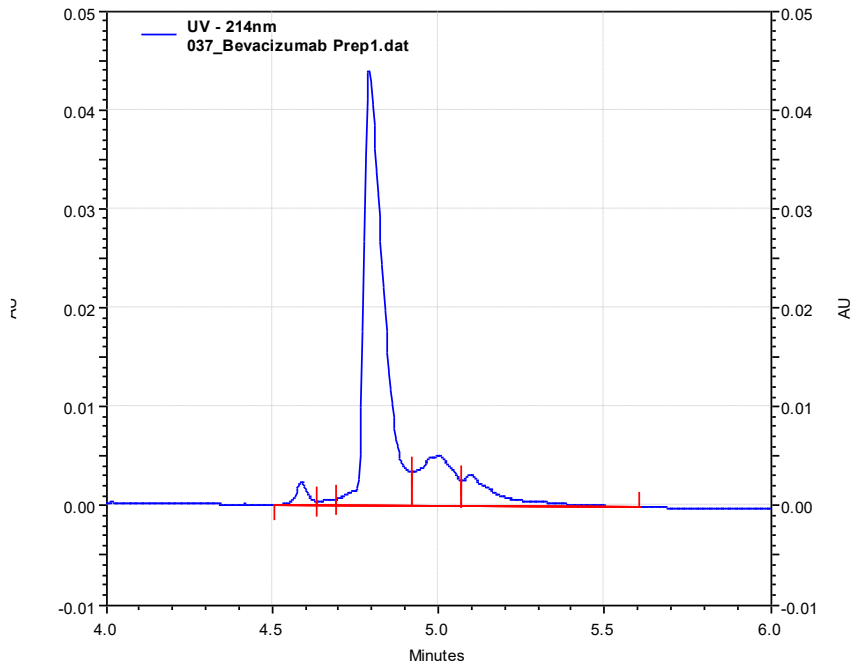


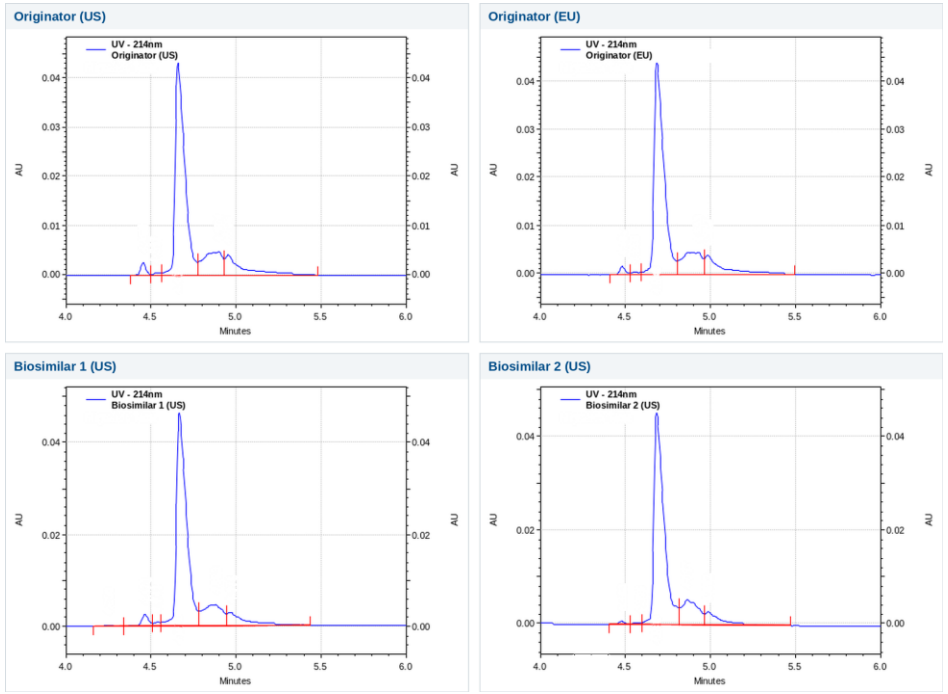
Sample	Acidic (%)	Main (%)	Basic (%)
USP Rituximab RS	**22.8**	**60.2**	**17.0**
Originator (US)	22.3	71.8	5.9
Originator (EU)	21.7	71.5	6.8
Biosimilar 1 (US)	23.7	65.6	10.7
Biosimilar 2 (US)	20.6	52.3	27.1

1. **RS data shown here are measured values**
2. **For detailed method please visit: AB Sciex website**

CZE: Bevacizumab

USP Bevacizumab RS



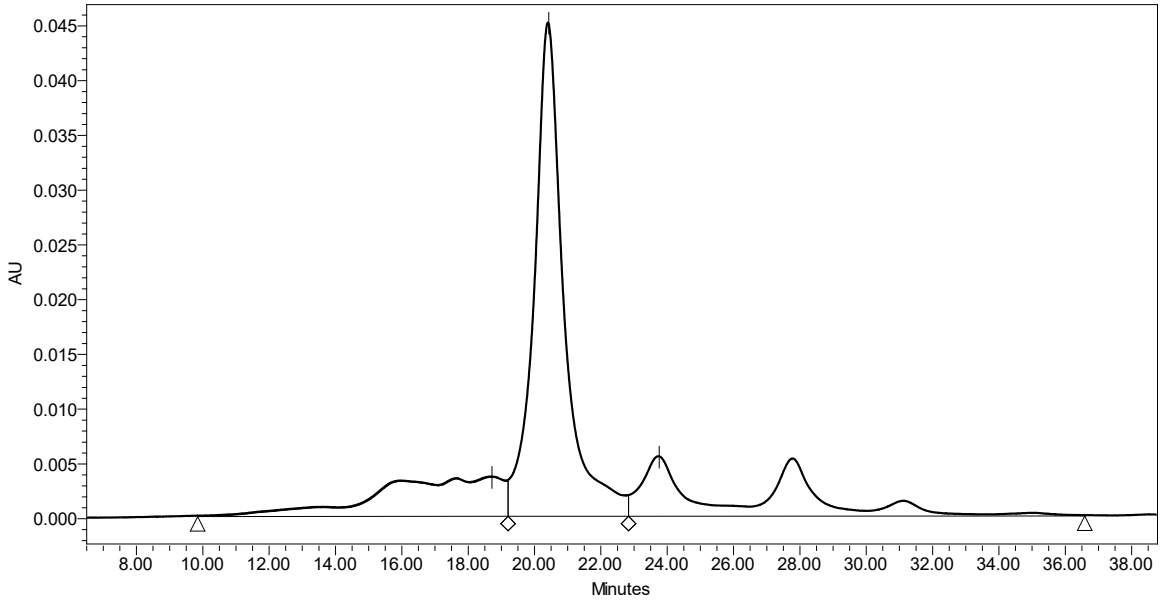


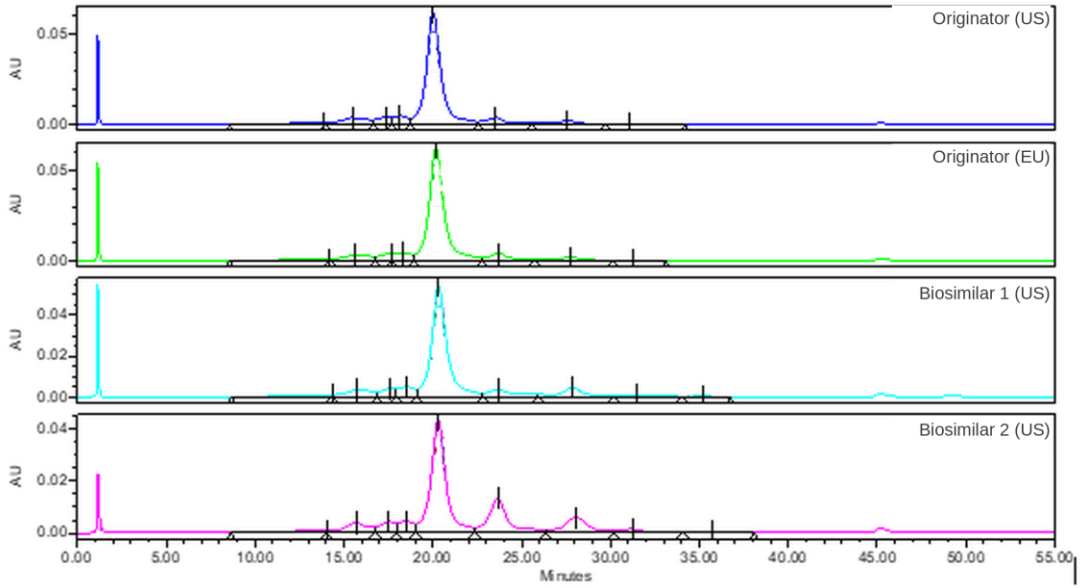
Sample	Acidic (%)	Main (%)	Basic (%)
USP Bevacizumab RS	**23.8**	**73.2**	**3.0**
Originator (US)	27.1	69.9	3.1
Originator (EU)	26.6	71.2	2.2
Biosimilar 1 (US)	22.2	74.0	3.9
Biosimilar 2 (US)	23.0	75.6	1.4

1. **RS data shown here are measured values**
2. **For detailed method please visit: AB Sciex website**

CEX: Rituximab

USP Rituximab RS



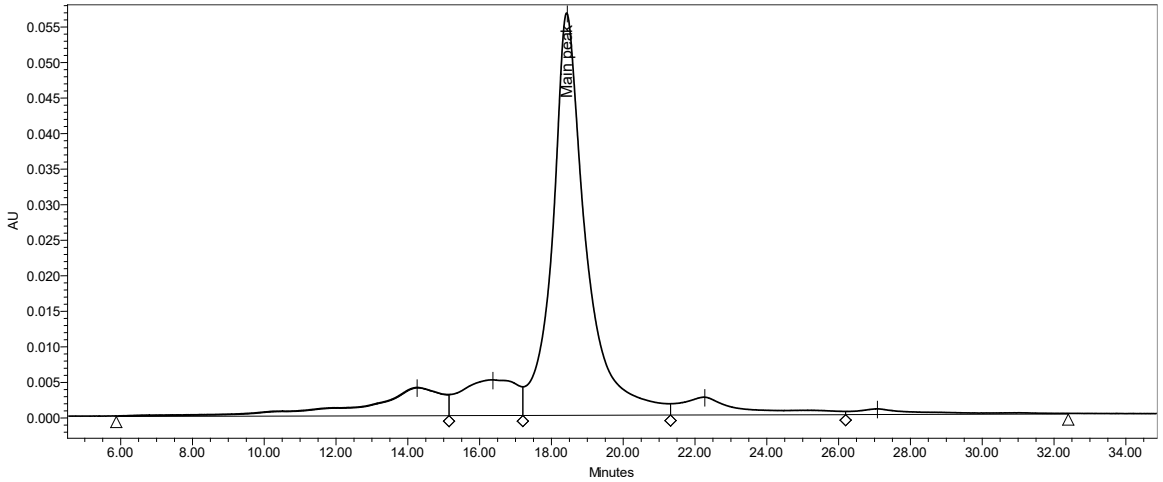


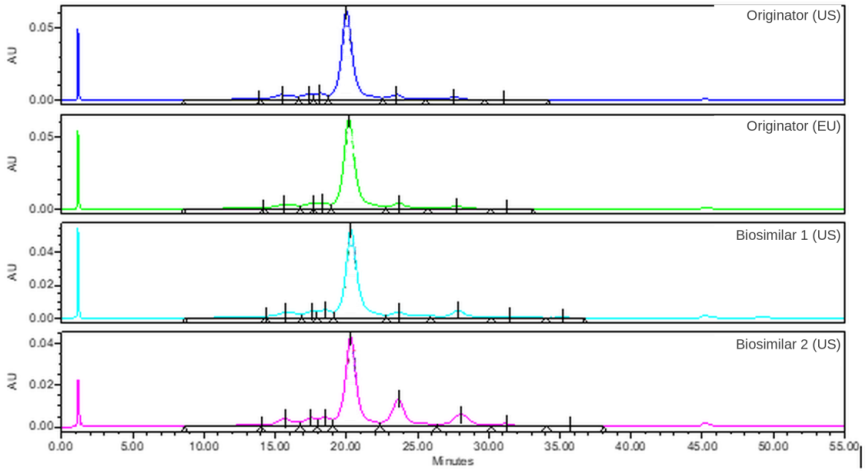
Sample	Acidic isoforms%	Main Peak%	Basic isoforms%
USP Rituximab RS	**20.04**	**57.63**	**22.33**
Originator (US)	19.11	70.61	10.29
Originator (EU)	17.72	71.77	10.51
Biosimilar 1 (US)	19.47	64.31	16.23
Biosimilar 2 (US)	18.51	49.52	31.97

1. **RS data shown here are measured values**
2. **For detailed method please visit: https://emergingstandards.usp.org/**

CEX: Bevacizumab

USP Bevacizumab RS





Sample	Acidic isoforms%	Main Peak%	Basic isoforms%
USP Bevacizumab RS	**21.38**	**71.49**	**7.14**
Originator (US)	25.83	68.43	5.75
Originator (EU)	24.55	70.42	5.04
Biosimilar 1 (US)	20.26	72.89	6.85
Biosimilar 2 (US)	21.66	73.13	5.21

1. **RS data shown here are measured values**
2. **For detailed method please visit: https://emergingstandards.usp.org/**

Comparison of Charge Variants Data

USP Rituximab RS

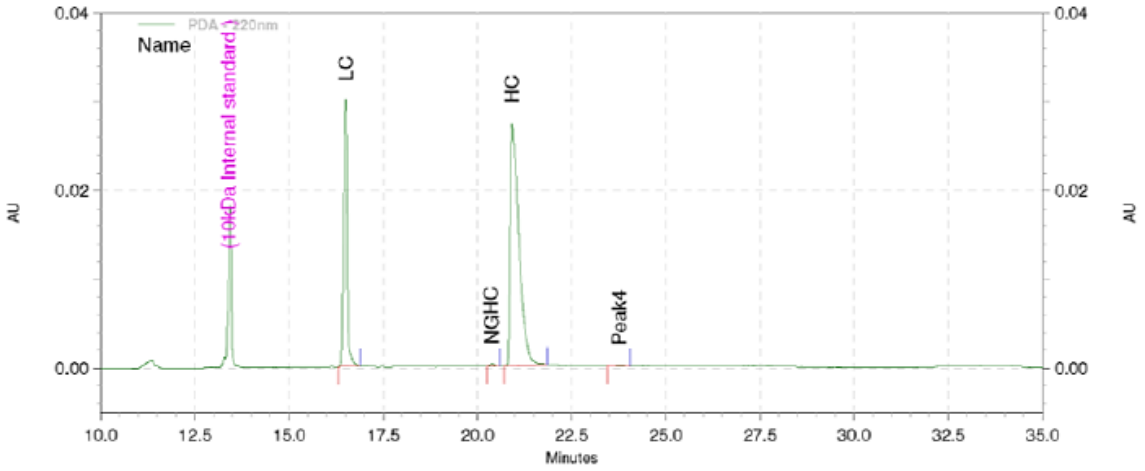
Sample	Main peak pI	Acidic (%)	Main (%)	Basic (%)
CEX	NA	20.04	57.63	22.33
cIEF	9.2	32	60	8
icIEF	9.2	38	54	8
CZE	NA	22.8	60.2	17.0

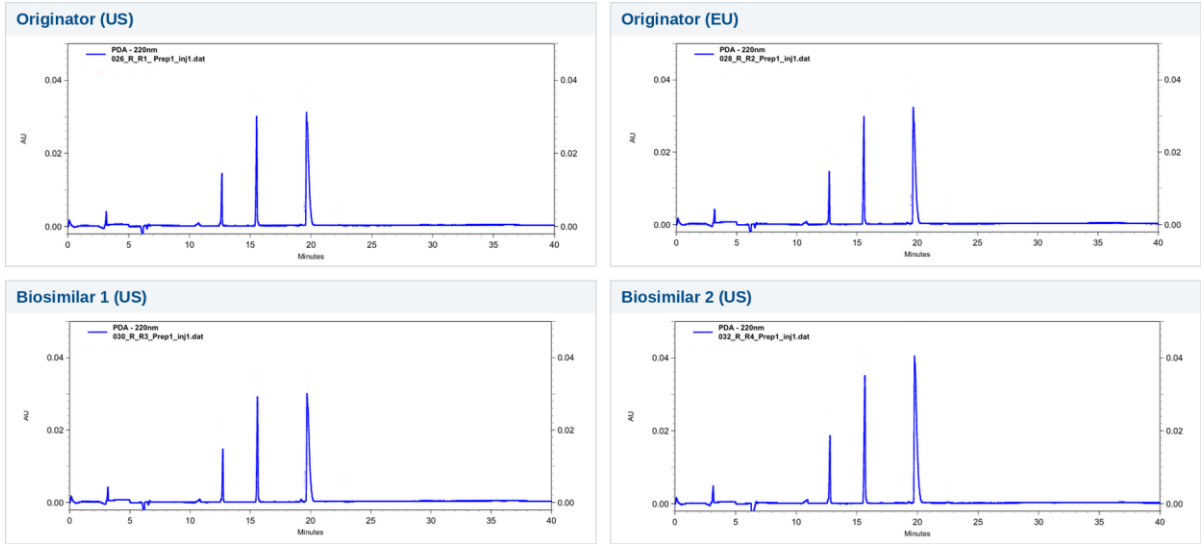
USP Bevacizumab RS

Sample	Main peak pI	Acidic (%)	Main (%)	Basic (%)
CEX	NA	21.38	71.49	7.14
cIEF	7.8	30	65	4
icIEF	7.9	28	67	4
CZE	NA	23.8	73.2	3.0

CE-SDS (Reduced): Rituximab

USP Rituximab RS



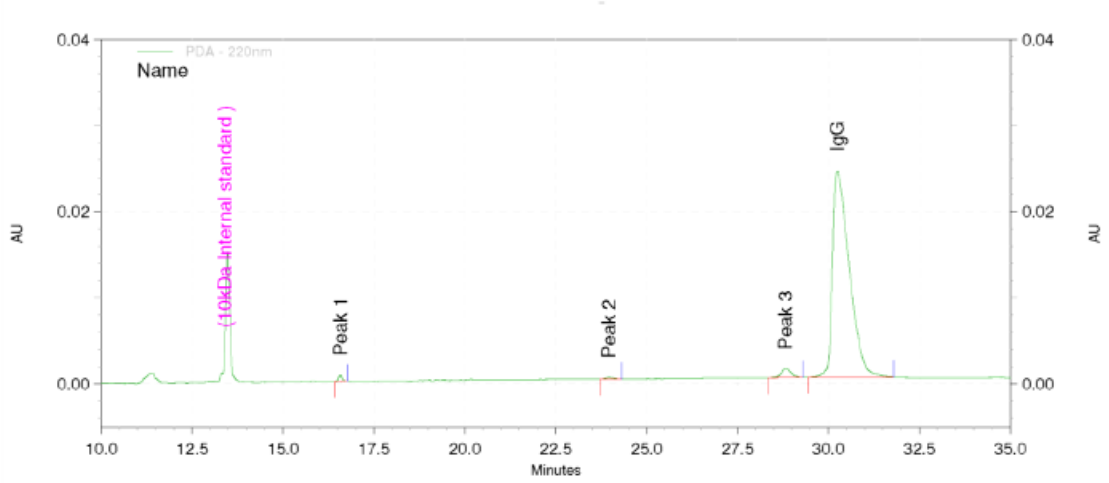


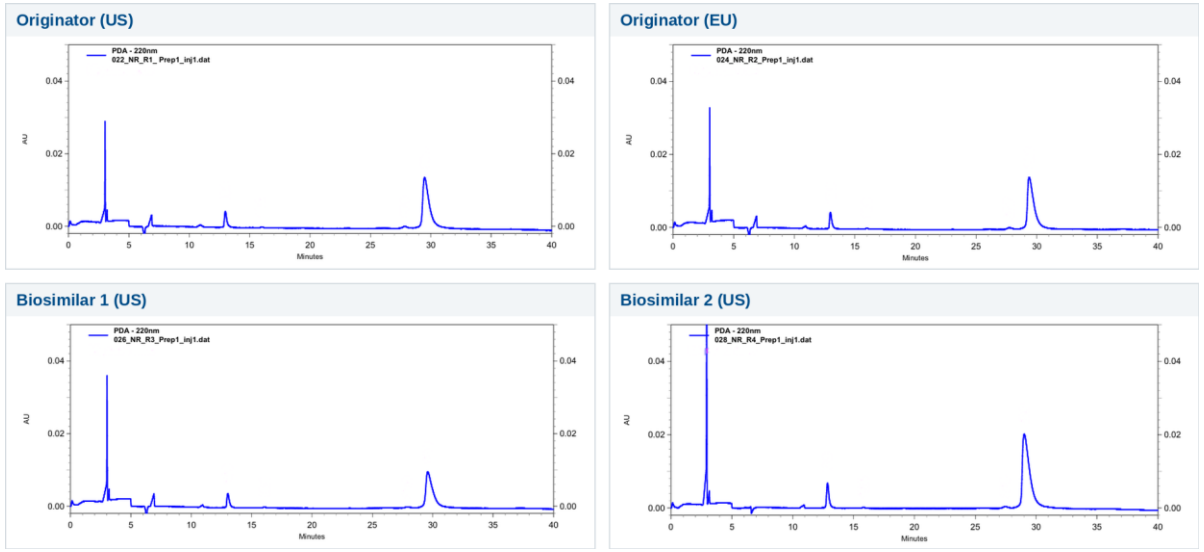
Species	USP Rituximab RS	Originator (US)	Originator (EU)	Biosimilar 1 (US)	Biosimilar 2 (US)
Light chain, LC (%)	**34.1**	33.1	33.2	33.4	33.2
Non-glycosylated HC, NG-HC (%)	**0.3**	0.3	0.4	0.7	0.3
Heavy chain, HC (%)	**65.4**	66.3	66.2	65.8	66.3
Unknown (%)	**0.3**	0.3	0.3	0.2	0.3

1. **RS data shown here are certificate values**
2. **For detailed method please visit: USP <129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies**

CE-SDS (Non-Reduced): Rituximab

USP Rituximab RS



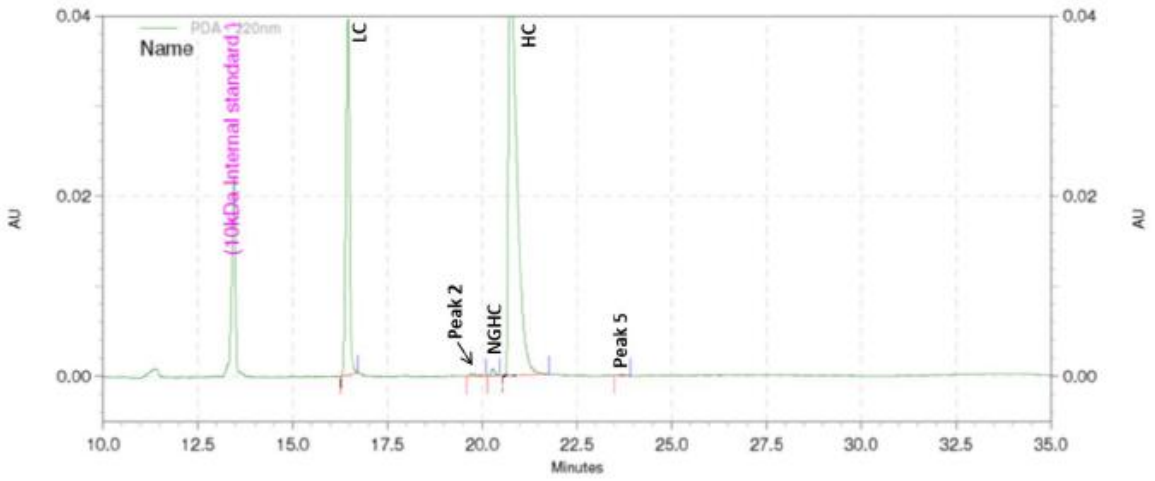


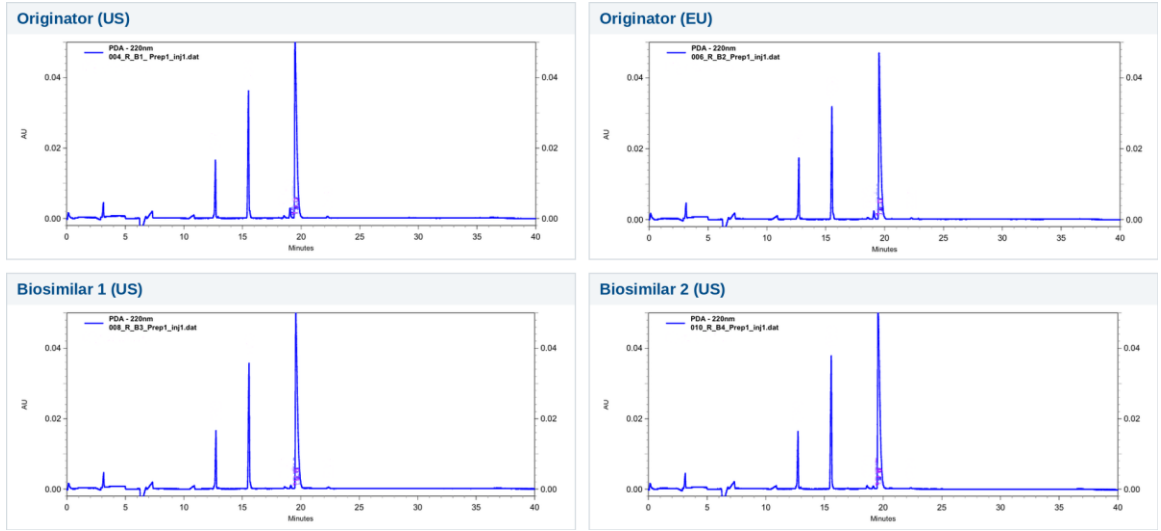
Species	USP Rituximab RS	Originator (US)	Originator (EU)	Biosimilar 1 (US)	Biosimilar 2 (US)
Intact IgG (%)	**95**	95.9	96.4	96.4	97.8
Non-glycosylated IgG, NG (%)	***not reported***	2.8	2.4	2.1	1.5
Unknown (%)	***not reported***	1.3	1.2	1.5	0.7

1. **RS data shown here are certificate values**
2. **For detailed method please visit: USP <129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies**

CE-SDS (Reduced): Bevacizumab

USP Bevacizumab RS



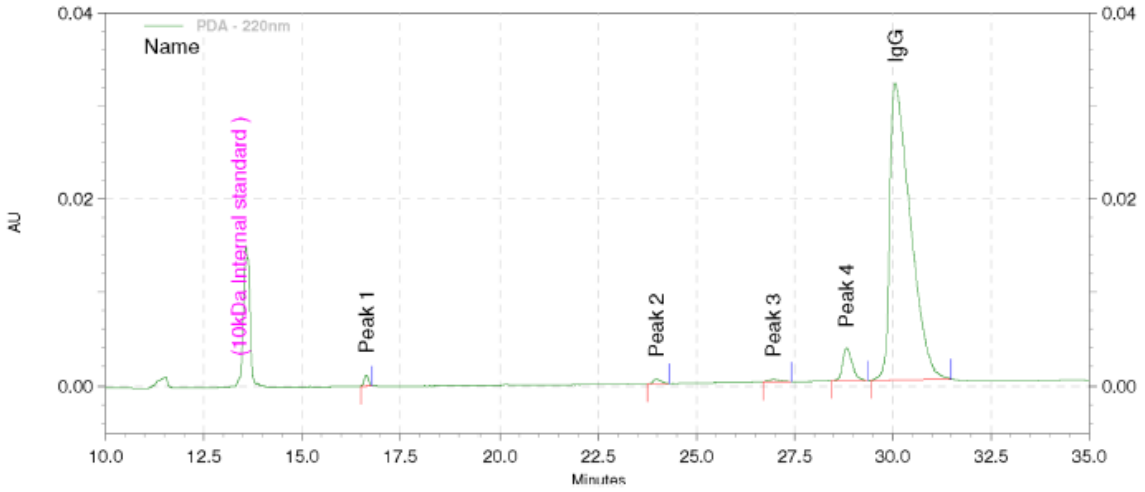


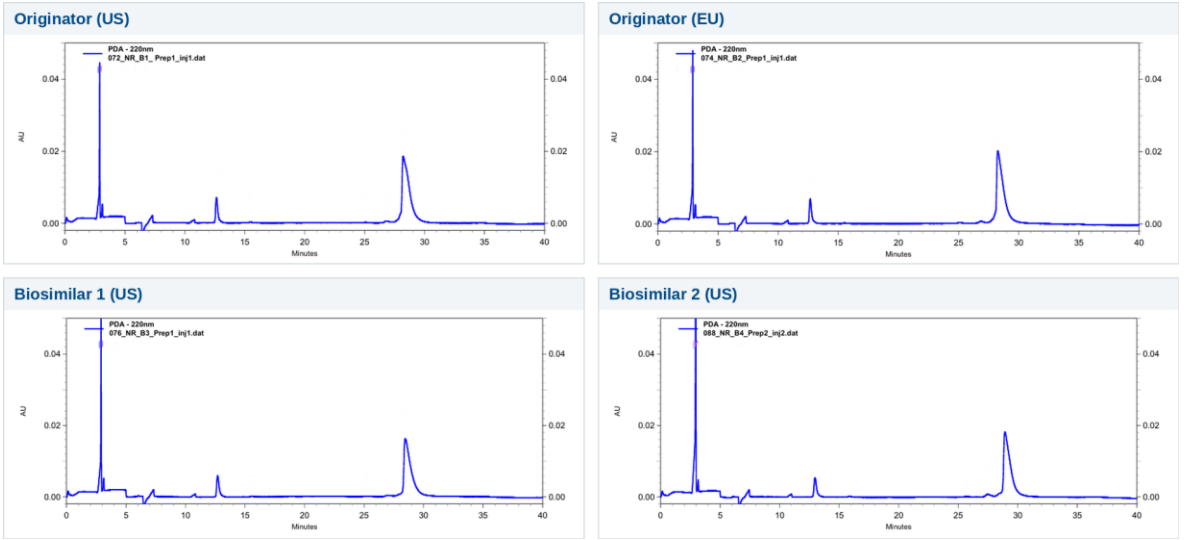
Species	USP Bevacizumab RS	Originator (US)	Originator (EU)	Biosimilar 1 (US)	Biosimilar 2 (US)
Light chain, LC (%)	**31.4**	31.1	31.1	31.2	31.3
Unknown 1 (%)	**0.4**	0.6	0.7	0.6	0.6
Non-glycosylated HC, NG-HC (%)	**0.6**	1.9	1.9	0.6	0.5
Heavy chain, HC (%)	**67.6**	66.0	65.9	67.3	67.4
Unknown 2 (%)	**0.1**	0.5	0.4	0.4	0.2

1. **RS data shown here are certificate values**
2. **For detailed method please visit: USP <129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies**

CE-SDS (Non-Reduced): Bevacizumab

USP Bevacizumab RS





Species	USP Bevacizumab RS	Originator (US)	Originator (EU)	Biosimilar 1 (US)	Biosimilar 2 (US)
Intact IgG (%)	**92**	97.5	96.8	97.2	96.7
Non-glycosylated IgG, NG (%)	***not reported***	1.4	1.8	1.5	2.2
Unknown 1 (%)	***not reported***	0.5	0.9	0.8	0.7
Unknown 2 (%)	***not reported***	0.5	0.5	0.5	0.4

1. **RS data shown here are certificate values**
2. **For detailed method please visit: USP <129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies**



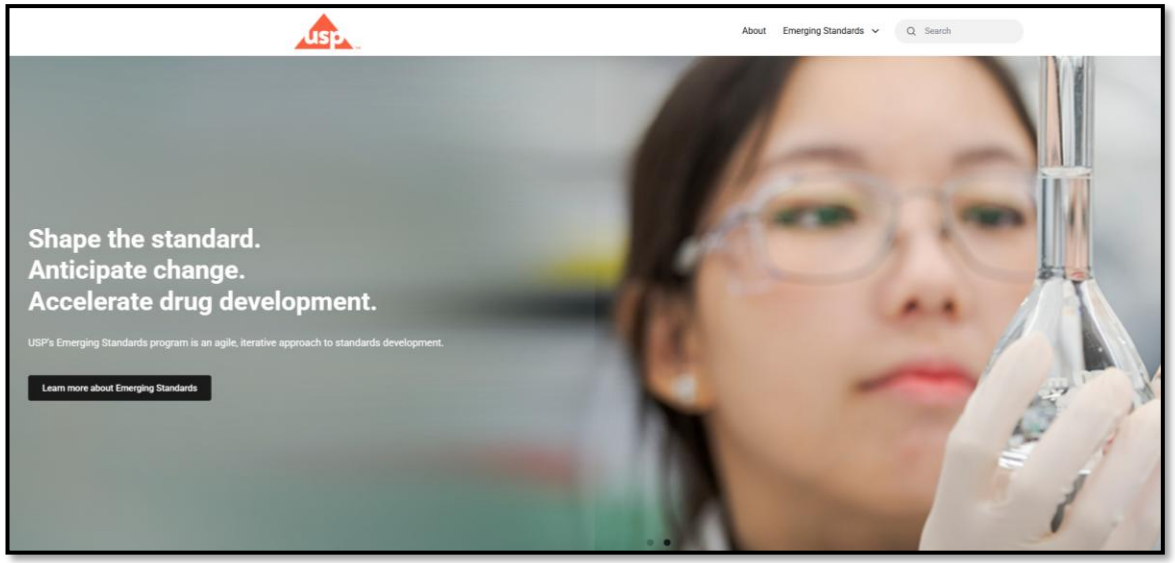
Conclusion

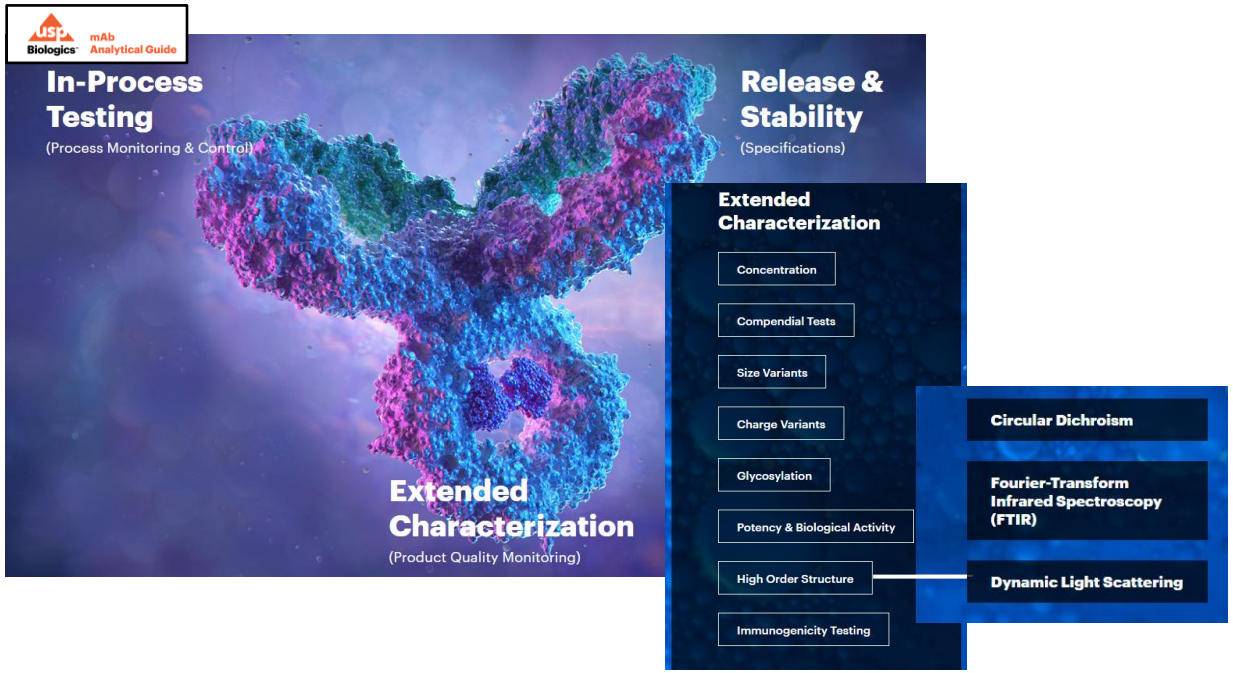
Summary

Capillary electrophoresis gives a practical, high-resolution toolkit for biosimilar mAb quality assessment

- Charge and size heterogeneity are core quality attributes for mAbs, and CE resolves both with small sample amounts and short run times
- cIEF/icIEF, CZE, and CEX are complementary rather than interchangeable: each separates on a different principle, and peak assignments should be interpreted within the method used
- Well-characterized reference standards anchor the comparison and give the results a consistent point of reference across labs and over time
- Together these methods help support a fit-for-purpose analytical package for biosimilar development, comparability, and lifecycle management

Resources for mAbs and Therapeutic Proteins





Emerging Standards

- Concept Note
- Rituximab
- Bevacizumab
- Epoetin
- Interferon beta-1a

Other USP Resources

- Reference Standards
- Documentary Standards
- USP Educational Contents
- Other Resources

www.usp.org/biologics

mAb Analytical Guide

Questions

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Thank you



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